REMARKS

Claims 4-24 were pending in this application when last examined. Claims 4 and 22-24 have been amended and new claims 25-27 have been added. Support for the amendments can be found in the specification. Support for claims 25-27 can be found in the specification, for example, at page 5, lines 15-22; page 7, lines 27-31; page 8, lines 10-15; and page 10, lines 19-27. No new matter has been added.

CLAIM REJECTION - 35 USC § 102

At page 2, the Advisory Action maintains the rejection of claims 4-6, 10-14, 16-18 and 22 under 35 U.S.C. § 102(b) as being anticipated by HINDS et al. (Enhanced gene replacement in mycobacteria, Microbiology, 1999, Vol. 145: 591-527). Applicants respectfully traverse the rejection.

The present application relates to the finding that a replication competent DNA molecule treated with a mutagenic agent that blocks its replication and transformed into a prokaryotic or eukaryotic cell, undergoes much higher levels of homologous recombination with the genome of the cell, than an untreated DNA molecule. Thus, the treated DNA molecule is a far superior agent for transforming prokaryotic or eukaryotic cells by targeted homologous recombination. This method overcomes some of the limitations associated with previous methods of transforming a

prokaryotic or eukaryotic cell by targeted homologous recombination.

As described in the present specification, the increased level of homologous recombination is likely due to the generation of highly "recombinogenic" ends resulting from partial replication of the mutagenic agent treated DNA molecule in the target cell. These highly recombinogenic ends remain active as the replication of the DNA molecule cannot become complete due to the mutagenic and replication blocking substance. (See, page 4, lines 8-31).

As such, claim 4 is directed to a method for *in vitro* insertion of a nucleic acid of interest within a predetermined target nucleotide sequence present in a chromosome of the cell genome. The method includes in part:

a) providing a DNA vector comprising the nucleic acid of interest, the vector being replication competent in the target cell;

b) contacting the DNA vector with a mutagenic agent blocking intracellular DNA replication of the DNA vector to produce a modified DNA vector;

c) transfecting the target cells with the modified DNA vector under conditions wherein replication of the modified DNA vector commences and insertion of the nucleic acid of interest within the predetermined target nucleotide sequence occurs.

HINDS fails to teach or suggest such a method. HINDS describes the development of an assay to study homologous recombination (HR) in *Mycobacterium*. Among their studies, HINDS uses plasmid vectors treated with UV radiation (pRAM4) and so called "suicide vectors" (e.g., pY6002). The Office Action, however, appears to misinterpret the methodology of HINDS.

For instance, the recombination assays using the pRAM4 plasmid are based upon intraplasmid recombination events (see, for example, page 521, 2nd column). What the authors in HINDS are looking for when using the pRAM4 plasmid is the loss or retention of a kanamycin resistance cassette positioned between two parts of a hygromycin resistance cassette. This means that the non-recombined pRAM4 plasmid will give rise to kanamycin resistant/hygromycin sensitive colonies and the recombined pRAM4 plasmid will give rise to kanamycin sensitive/hygromycin resistant colonies. The HINDS reference does not study the transfer of nucleic acid from the plasmid to a chromosome of the host cell. HINDS discloses an intraplasmid HR assay, i.e., an assay of HR events within the same plasmid, using the pRAM4 plasmid.

In these studies, HINDS fails to teach or suggest any method that includes "insertion of a nucleic acid of interest initially included in a DNA vector, within a predetermined target nucleotide sequence present in a chromosome" of the cell genome

as recited in the presently claimed method. Indeed, HINDS recognizes that "factors causing an increase in intraplasmid recombination might not be effective in improving recombination between plasmid and chromosome ..." (see, page 523, 2nd column, 2nd paragraph).

HINDS does further study recombination between plasmid and chromosome using, for example, "gene replacement" vectors pY6002 and pSYCH09 (see, page 523, 2nd column to page 524). In distinction from the presently claimed method, however, HINDS utilizes "suicide vectors" i.e., a plasmid that cannot replicate in a particular host (http://www.everythingbio.com). In this regard, HINDS clearly states that "four genes in three species were successfully knocked-out using non-replicating DNA pretreated with with alkali, UV or in an SS form" (see, Abstract, emphasis added).

The teachings of HINDS are completely in line with the existing prior art. That is, if one of ordinary skill in the art intends to introduce a nucleic acid sequence into the genome of a target cell by homologous recombination, then one would use a non-replication competent DNA vector (i.e., suicide vector). Because the likelihood of a homologous recombination event is so small compared to the likelihood of a stable transfection of the target cell by a replication competent vector, any homologous recombination events that do occur will be virtually

undetectable, unless such a non-replicating suicide vector is utilized.

HINDS also fails to teach or suggest the use of a "mutagenic agent blocking intracellular DNA replication" of the DNA vector, as featured in the presently claimed method. In their studies, HINDS analyzes several parameters to determine their effects on homologous recombination. This included treatment of the DNA with alkali and boiling (denaturing) and with UV irradiation. Of significance to the present application, the UV treatment disclosed in HINDS is utilized to cause DNA damage and stimulate *recA* or other DNA repair systems (see, page 525, 2nd column, first paragraph). HINDS fails to teach or suggest or analyze whether any blocking of DNA replication occurs, as featured by mutagenic agent recited in the presently claimed method.

For all of these reasons, HINDS fails to teach or suggest, and does not anticipate claims 4-6, 10-14, 16-18 and 22. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CLAIM REJECTION - 35 USC § 103

At page 5, the Advisory Action maintains the rejection of claims 4-21 and 23-24 under 35 U.S.C. § 103(a) as being

unpatentable over HOEIJMAKERS et al. (US 2003/0124605) in view of HINDS. Applicants respectfully traverse the rejection.

First, as detailed in the above remarks, HINDS fails to teach or suggest any method having the combination of features as recited in the present claims.

Second, the Office Action appears to have misinterpreted HOEIJMAKERS. HOEIJMAKERS relates to methods to monitor the levels of HR23 protein binding partners in cells following exposure to a DNA modifying agent (i.e., a mutagen). The cells used in this method were engineered to have altered expression of HR23A and/or HR23B. For HR23A, this was achieved by targeted gene disruption in accordance with the scheme shown in Figure 1. The strategy outlined in Figure 1 and detailed at page 9, paragraphs 108-115, is a known homologous recombination gene alteration strategy. This strategy involves the generation of a mHR23A targeting vector which has two portions of homology to exons II and exons VII/VIII of the HR23A gene and flanking a neomycin resistance cassette (see, Figure 1 and paragraph [0108]).

This non-replication competent targeting vector (a bacterial plasmid in a target mammalian cell) is then electroporated into the target cells. Following culturing and expansion of the initial target population, G418 resistant clones are selected based upon the presence of the neomycin resistance

cassette as a stable integrant. (See, Figure 1 and paragraph [0110]). Then, using these transformed cells, homozygous double mutant HR23A$^{-/-}$ B$^{-/-}$ mice transgenic mice were created (see, paragraph [0112]).

In this series of steps, the vector constructs are not replication competent in the target (eukaryotic) ES cell. The final construct, pG5M23Ag30, is pGEM-9Zf(-) based (Promega) and comprises only a prokaryotic origin of replication. Thus, like HINDS, HOEIJMAKERS also fails to teach or suggest the use of a DNA vector that is replication competent in the target cell.

In another series of HOEIJMAKERS experiments, for HR23B, a full length cDNA of this gene was inserted into the pSLM vector and then transfected into Double-Knockout Mouse embryo fibroblasts, together with a separate vector comprising a full length cDNA of the hXPC-GFP fusion protein. The resulting transformed cells were then selected for stable transformants based upon puromycin resistance (see, paragraphs [0129] and [0130]).

In distinction from the presently claimed methods, the nucleic acids of interest do not insert within a predetermined target nucleotide sequence present in the cell genome. The transforming DNA is randomly inserted into the target cell genome as per the normal transfection protocol.

Also, at no point prior to or following the introduction of any of the targeting vectors into the target cell was the vector exposed to any mutagen such as UV or a chemical mutagen. Such exposure only occurred after the stable clones resulting from the transfection event had been selected for and isolated. The exposure of the cells to a mutagen was to study the response of HR23 protein binding partners in cells following exposure to a DNA modifying agent.

For all of these reasons, the combination of HOEIJMAKERS and HINDS fails to teach or suggest, and fails to render obvious, the methods of present claims 4-21 and 23-24. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CLAIM REJECTION - 35 USC § 112

At page 10, the Advisory Action maintains the rejection of claims 4-24 under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse the rejection.

Currently amended claim 4 further clarifies the intended subject matter and addresses the issues noted in the Office Action. In particular, amended claim 4 indicates that a replication competent DNA vector is a) provided, b) contacted with mutagenic agent, and c) transfected into cells.

First, one of ordinary skill would understand this series of steps.

Second, whether the sequence of steps occurs in any particular order is not relevant to a determination of claim indefiniteness in this instance. The law under 35 U.S.C. § 112, second paragraph, requires that "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986). The Office Action appears to have rejected claim 4 by alleging that breadth alone (the sequence of steps) gives rise to the indefiniteness. As pointed out, "[b]readth of a claim is not to be equated with indefiniteness." *In re Miller*, 169 USPQ 597 (CCPA 1971).

Accordingly, Applicants request reconsideration and withdrawal of the rejection.

CONCLUSION

Entry of the above amendments is earnestly solicited. Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any deficiency or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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